Bull. Inst. Zool., Academia Sinica **1:** 1-8 (1962)

THE EFFECT OF AUREOMYCIN ON THE REPRODUCTION IN ALBINO MICE[1]

CHIH-YÜN HSÜ[2], CHUNG-LING CHU[3] AND HSÜ-MU LIANG[4]

Received for publication April 16, 1962

While the use of antibiotics in the control of infection is well known, its application as growth stimulant in animal husbandry raises quite a few questions of considerable interest. Antibiotics, mainly aureomycin, terramycin and penicillin, when added in minute amount to the feeds of young animals improved their growth rate (1, 2, 3). This growth stimulating effect was thought to be the result of suppression of pathogenic or subclinical bacteria with an augmentation of the vitamin-B producing micro-organisms in the intestinal microflora (3).

This antibacterial concept of antibiotics in growth promotion received support from work by Carpenter (4), Carpenter and Larson (5) and Davey *et al.* (6). They all reported that when the diet of the sows was supplemented with aureomycin, the birth weight of the new born pigs was not affected. Carpenter and Larson believed that the intrauterine sterile environment rendered the action of aureomycin non-manifest, resulting in no growth promotion of the fetal pigs.

On the other hand, the indifferent reaction of the developing pig embryos to the aureomycin treatment may be attributed to the fact that sows are indecidual animals with epitheliochorial type of placenta (7, 8) which consists of six layers, the maximum possible, and the pig would have a transplacental exchange rate among the lowest in mammals. This might hinder the passage of aureomycin, a rather large molecular chlortetracycline, and hence no or very little aureomycin could reach the fetuses to produce any stimulating effect. Carpenter and Larson found that neither aureomycin nor penicillin was transferred across the placenta of the sow (5).

The placental type of the rat is not far from that of man and belongs to the same category of haemochorial type which is composed of three layers only. Elliott and Whitehill showed that when pregnant rats were fed diets containing aureomycin the antibiotic was demonstrable in the fetal tissues, but they did not notice any significant increase in litter size or birth weight of the young born to the treated mothers (9).

In order to test the validity that aureomycin does not affect the growth rate of germ-free organisms, the embryos of the mouse, which is another animal with haemochorial type of placenta, were chosen for the present observation.

MATERIALS AND METHODS

Albino mice, NIH strain, about two months old were used. The first litters of the animals were discarded. Then after mating pregnant females were divided into two groups at random. The experimental group received two intraperitoneal injections daily, each of 0.1 mg of aureomycin in 0.5 ml of distilled water beginning from the first day of gestation to the end. This dosage was estimated from the fact that the nutritional dose of antibiotics by oral feeding to chicks, swines and other domestic animals was of a very minute order, being 10 to 100 ppm of the basal ration. A pilot experiment, in which five pairs of the same strain of mice were allowed to take food and water freely during a period of 10 days, showed that the average daily food intake per mouse was 3.6 g. In order to make it 100 ppm of the food, aureomycin needed for injection should

1 This study was supported in part by a grant from China Medical Board of New York, Inc.

2 Professor, Department of Biomorphics, National Defense Medical Center.

3 Assistant, Department of Biomorphics, National Defense Medical Center. Now, research assistant, Institute of Zoology, Academia Sinica.

4 Professor and head of Department of Biomorphics, National Defense Medical Center, and director of Institute of Zoology, Academia Sinica.

be 0.36 mg per mouse per day. As the absorption of aureomycin by the peritoneal route is usually quicker and more than that by the oral one, 0.2 mg of aureomycin per mouse per day was therefore given. The fact that parenteral administration was adopted was to rule out the direct action of aureomycin on intestinal bacteria of the mother. The control animals received no sham injection.

Both groups were fed grain mixture with 1.5% of cod liver oil, green vegetables, and water *ad libitum*. All pregnant females were caged individually under the same laboratory environment.

During gestation period the following observations were made:

1) Daily record of maximal and minimal room temperature.
2) Daily measurement of the maternal body weight.
3) Length of the gestation period.
4) Litter size.
5) Birth weight of the young.
6) Number of still-birth.
7) Number of premature termination of pregnancy (resorption of embryos).

The experiment was repeated three more times, using fresh females on each occasion, during different months of the year. There were 29, 31, 24, and 19 female mice used in the first, second, third, and fourth experiment respectively.

RESULTS AND DISCUSSION

The four experiments were carried out consecutively one after another with an interval of about ten days between experiments. The reproduction performance was summarized in Table I.

Premature Termination of Pregnancy and Still-birth

Two daily injections during the whole period of gestation produced no harm both to mothers and their young since no difference could be made out between them and the controls, insofar as premature termination of pregnancy (resorption of embryos) and stillbirth were concerned (Tables II and III).

Litter Size

Litter size usually varies greatly with factors such as the strain, the age, the order of the litter and the general condition of the mother including function of the ovary, fertility of the germ cells and condition of the uterus. In the present experiments only the condition of the mother might be altered by aureomycin treatment. However no statistically significant difference was found in litter size between control and experimental groups of all four experiments. This result confirms the findings of Elliott and Whitehill on rat (9) and gives no indication of a possible relationship between aureomycin injection and the general condition of the mother.

When the litter size of various experiments was compared, that of both the control and the experimental groups of Experiment III was significantly larger than that of the respective groups of all the other experiments (Table IV).

Since the litter size was increased in both the control and the experimental mice of Experiment III, aureomycin treatment was apparently not involved. It is possible, however, that the litter size was related to the maternal body weight which had been shown to be highly correlated with the number of eggs ovulated (10). Therefore the original body weight of the mother before gestation was analyzed (Table V).

TABLE I

Summary of crude data on reproduction performance

Experiment		No. of pregnant mice	No. of litters	Av. litter size±S. E.	Av. gestation period ±S. E., days	Av. increase of maternal body wt. ±S. E., %	Av. birth wt. of the young ±S. E., g	Av. initial maternal body wt. ±S. E., g
I	Control	16	15	6.5±0.36	20.0±0.22	39.8±2.20	1.06±0.026	26.5±0.36
	Exptal.	13	12	5.9±0.48	19.6±0.38	45.4±3.57	1.11±0.030	
II	Control	14	11	6.5±0.55	19.6±0.26	44.3±3.37	0.97±0.045	24.8±0.48
	Exptal.	17	13	6.4±0.59	19.3±0.11	43.3±1.87	1.07±0.030	
III	Control	12	11	8.7±0.51	19.4±0.21	78.1±5.56	1.29±0.019	26.8±0.31
	Exptal.	12	8	9.2±1.03	19.7±0.16	77.0±5.18	1.31±0.068	
IV	Control	9	8	6.9±0.69	19.4±0.32	84.0±2.87	0.91±0.046	24.5±0.42
	Exptal.	10	10	6.2±0.80	19.3±0.15	87.6±3.40	1.10±0.042	

TABLE II

Comparison of premature termination of pregnancy between control and aureomycin-treated mice

Experiment	No. of termination of pregnancy	No. of litters	Total no. of pregnant mice	Percentage of termination of pregnancy
Control:				
I	1	15	16	
II	3	11	14	
III	1	11	12	
IV	1	8	9	
Total	6	45	51	11.8
Experimental:				
I	1	12	13	
II	4	13	17	
III	4	8	12	
IV	0	10	10	
Total	9	43	52	17.3
				P =0.81

TABLE III

Comparison of still-birth between control and aureomycin-treated mice

Experiment	No. of still-birth	No. of young alive	Total young	Percentage of still-birth
Control:				
I	9	98	107	
II	6	72	78	
III	6	96	102	
IV	7	55	62	
Total	28	321	349	8.0
Experimental:				
I	8	71	79	
II	6	84	90	
III	2	74	76	
IV	3	62	65	
Total	19	291	310	6.1
				P =0.32

TABLE IV

Comparison of litter size between control and auromycin-treated mice

Experiment	No. of litters	Av. litter size ±S. E.	"P" value (t-Test)		
			1*	2	3
Control:					
III	11	8.7±0.51			
IV	8	6.9±0.69	0.04		
I	15	6.5±0.36	<0.01	0.63	
II	11	6.5±0.55	<0.01	0.70	>0.90
Experimental:					
III	8	9.2±1.03			
IV	10	6.2±0.80	0.03		
I	12	5.9±0.48	<0.01	0.51	
II	13	6.4±0.59	0.02	0.79	0.53

*1: Probability between III & I, III & II, III & IV.
2: Probability between IV & I, IV & II.
3: Probability between I & II.

TABLE V

Comparison of initial maternal body weight of the four experiments

Experiment	No. of mice	Av. initiadl maternal bo y wt. ±S. E., g	"P" value		
			1*	2	3
III	19	26.8±0.31			
I	27	26.5±0.36	0.61		
II	24	24.8±0.48	<0.01	<0.01	
IV	18	24.5±0.42	<0.01	<0.01	0.59

*1: Probability between III & I, III & II, III & IV.
2: Probability between I & II, I & IV.
3: Probability between II & IV.

It is obvious from the above table that the initial maternal body weight of Experiments I and III were significantly larger ($P<0.01$) than that of Experiments II and IV. However, only mothers in Experiment III produced larger litter. This point deserves further attention.

Gestation Period

The gestation period was also not observed to be affected by aureomycin injection as no significant difference in each experiment was found between control and aureomycin-treated mice. Since the length of gestation could be a function of the mother besides the genetic factors, it is assumed again that aureomycin injection was not likely to be involved to influence the pregnant mothers themselves.

Increase of Maternal Body Weight

The increase of maternal body weight during gestation period is usually correlated positively with the litter size. In order to eliminate the factor of variation of the initial body weight, the increase of maternal body weight was taken in terms of per cent increment above the initial figure and analyzed for covariance. Table VI gives the results of such an analysis of the pooled data. It indicates that the correlation coefficients (r) between increase of maternal body weight and litter size of all four controls and experimentals were significantly positive, as usually did. It is to be noted that the comparison of the adjusted mean increase of maternal body weight between controls and experimentals is null, and the comparison of regression coefficients (b) is

TABLE VI

Comparison of the increase of maternal body weight between control and aureomycin-treated mice

Experiment	No. of litters	"r" between increase of body wt. and litter size	Adjusted mean increase ±S. E., %	"F" value of adj. mean increase	"F" value of "b" of mean increase on litter size
Four controls	45	0.538(P<0.01)	58.0±3.34	0.28(P>0.05)	0.24(P>0.05)
Four exptals	43	0.350(P<0.05)	60.4±3.28		

so too. The analysis gives no support to a significant shift of auremycin effect on the increase of maternal body weight.

Birth Weight of the New Born Mice

Aureomycin most probably exerted no influence on pregnant mice, as the analysis of premature termination of pregnancy, still-birth, litter size, gestation period and the increase of maternal body weight revealed. But situation changed when birth weight of the young born to control and experimental mothers was compared. This is in accordance with the established fact that antibiotic growth effect is observed in young animals only.

As there usually exists a close relationship between birth weight and litter size, the method of analysis of covariance is therefore again adopted in Table VII. Since the initial maternal body weight of the four experiments was not the same, the comparison was made on individual experiment. The data in Table VII indicate that the growth stimulating action of aureomycin is demonstrable in experimental groups II, III and IV, by a combination of three methods of analysis. 1) The correlation coefficient (r) between birth weight and litter size was positive in control groups of Experiments II and III, in significant contrast to the negative coefficient figures (P< 0.05) of the respective experimental groups. 2) The regression coefficients (b) of birth weight on litter size in controls and experimentals of Experiment III were significantly different, namely, the slopes of the regression lines of the two groups were not the same. 3) when the adjusted mean birth weight was compared, a significant difference was found between control and experimental animals in Experiment IV, *i.e.*, the birth weight of the young born to the experimental mice was increased.

When a difference of birth weight exists between the control and experimental groups, the maternal body weights of the two should differ likewise. This was, however, not demonstrated in the present case, indicating that the gain in birth weight of the new born was achieved at the expense of the mother as indicated by Table VI and Fig. 1 which is a plot of the increase of maternal body weight against gestation in days. The figure reveals on the whole a more rapid increase in maternal body weight about five days before parturition. During this critical period the demand of the fetuses on the mother should be much greater than it had been before, thus putting the mother under strain.

TABLE VII

Comparison of birth weight of the young born to control and aureomycin-treated mice

Experiment	No. of litters	"r" between birth wt. and litter size	Adjusted mean birth wt. ±S. E., g	"F" value of adj. mean birth wt.	"F" value of "b" of birth wt. on litter size
I Control	15	-0.049(P>0.05)	1.06±0.024	1.78(P>0.05)	0.02(P>0.05)
Exptal.	12	-0.130(P>0.05)	1.11±0.033		
II Control	11	0.254(P>0.05)	0.97±0.044	3.78(P>0.05)	3.64(P>0.05)
Exptal.	13	-0.586(P<0.05)	1.07±0.026		
III Control	11	0.184(P>0.05)	1.29±0.019	1.21(P>0.05)	5.41(P<0.05)
Exptal.	8	-0.735(P<0.05)	1.31±0.060		
IV Control	8	-0.311(P>0.05)	0.91±0.043	6.15(P<0.05)	0.01(P>0.05)
Exptal.	10	-0.442(P>0.05)	1.10±0.037		

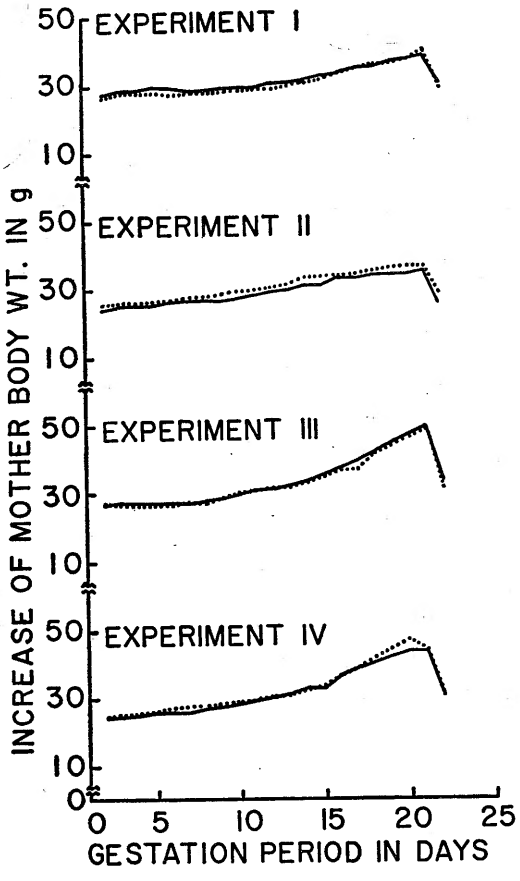

Fig. 1. Growth curves of the increase of maternal body weight of the four experiments. Solid lines represent controls and dotted lines the experimentals.

Since the four experiments were not carried out simultaneously, the effect of environmental temperature variation should be examined. Fig. 2 depicts fluctuation of maximal and minimal room temperature during gestation. When the critical period, as marked out by the two arrows in Fig. 2, was studied on the temperature curves, it was apparent that the maximal and the minimal temperature varied very much in Experiments III and IV, but only slightly in Experiments I and II. Furthermore, whereas the temperature dropped continuously within this period in Experiment III, it rose stiffly in Experiment IV.

When the birth weight of the new born was analyzed with respect to temperature fluctuation in this period, it was obvious that firstly the birth weight of the control and experimental new born (Experiments I and II) showed no significant difference (Table VII) with slight temperature variation; secondly, with a continuous drop of temperature (Experiment III) both the control and experimental new born were the heaviest among all (Table VIII); and thirdly, with a stiff rise of temperature (Experiment IV) the new born of experimental mice showed a significant increase of birth weight over that of the controls (Table VII).

It can be off hand mentioned from the above result that a gain in birth weight of both the control and experimental youngs in Experiment III might be the result of a favorable temperature (a continuous fall from high to optimal) which made the pregnant mice eat more, thus allowing the fetuses to obtain more nutriment for development from an otherwise overtaxed mother during this period. Whereas in Experiment IV a sudden rise of temperature was certainly a burden before acclimatization, and would put the already strained mice under an adverse condition. This might break the barrier for aureomycin to reach the fetuses and stimulated them to grow heavier.

It is very possible that aureomycin action on development might be a continuous one lasting quite a length of period, not necessarily confining to the last few days in gestation. However, in the present experiments the embryos were not examined for such purpose. Therefore it remains a question as to when the growth simulating action of aureomycin begins during mouse development.

The increase of birth weight, the change of correlation coefficient between birth weight and litter size from positive to negative, and the shift of slopes of the regression lines of birth weight on litter size (Table VII) all indicate that development was stimulated in experimental groups in view of the finding of aureomycin transfer across rat placenta (9). They lend no support to the antibacterial view of aureomycin action in growth promotion. The possibility that an improved growth rate or the improved general condition of the pregnant mice induced by aureomycin may indirectly favor the embryos is likely non-existent, for antibiotic growth effect is obtainable only in young and immature animals. Further more the present results indicated that the general condition of the experimental pregnant mice was not improved.

The current postulation of antibiotic growth-promoting effect, based on antibacterial action upon intestinal microbes, therefore needs further support, particularly when the great variation of bacterial counts made on the intestinal content of animals fed antibiotic ration is taken into consideration (3).

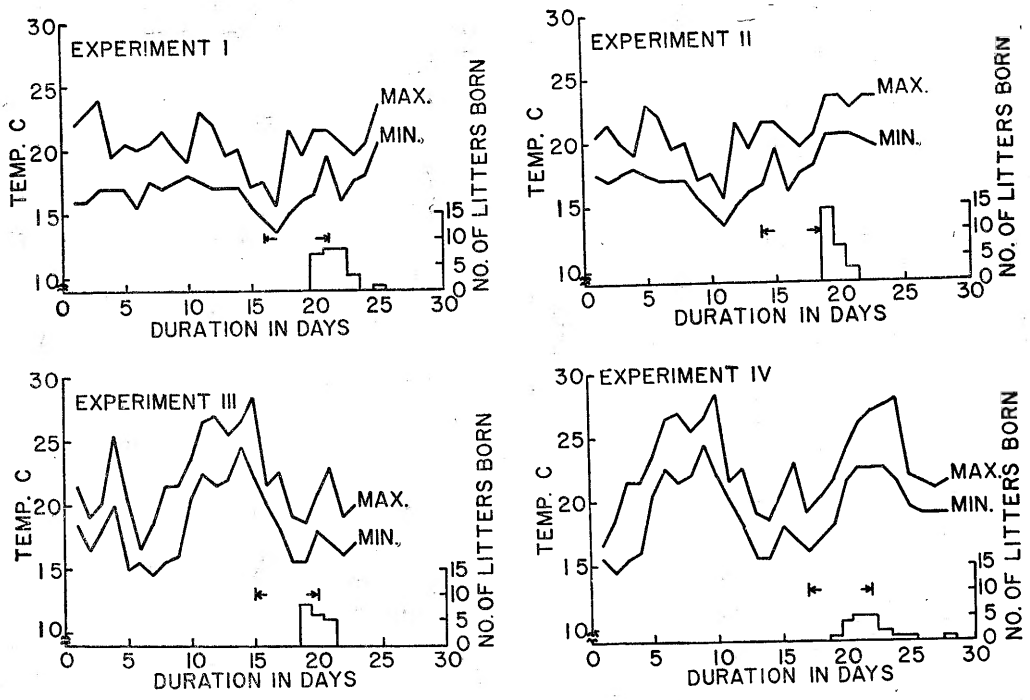

Fig. 2. Fluctuation of maximal and minimal room temperature and the histogram representing the frequency distribution of litters born during the gestation period of the four experiments.

TABLE VIII

Comparison of birth weight of the young of the four experiments

Experiment	No. of litters	Adj. mean birth wt. ±S. E., g	"P" value (t-Test)		
			1*	2	3
Control:					
III	11	1.29±0.019			
I	15	1.06±0.024	<0.01		
II	11	0.97±0.044	<0.01	0.07	
IV	8	0.91±0.043	<0.01	<0.01	0.44
Experimental:					
III	8	1.31±0.060			
I	12	1.11±0.033	<0.01		
II	13	1.07±0.026	<0.01	0.44	
IV	10	1.10±0.037	<0.01	0.89	0.56

* 1: Probability between III & I, III & II, and III & IV.
2: Probability between I & II, I & IV.
3: Probability between II & IV.

In fact, evidences which discourage the antibacterial view of antibiotic growth promoting effect are accumulating. Hester *et al.* reported that injection of aureomycin in dairy calves resulted in an increased growth, but no antibiotic was found in the lumen of small intestine (11). Nickell claimed that antibiotic treatment stimulated plant growth in virus tumor in tissue culture, in germinated seeds, and in germinated seeds with subsequent growth in soil (12). Barber and

co-workers demonstrated the additive effect on growth and feeding efficiencies, when aureomycin in combination with diethylstilbestrol and L-thyroxine was fed to growing pigs (13). Grant found a small but definite increase of the uptake of I^{131} by the thyroid when aureomycin was fed to rats (14). Hsü showed when tadpoles were fed food supplemented with aureomycin, their rate of metamorphosis was hastened (15). The same author and co-workers found that another antibiotic, penicillin, gave no growth-stimulating effect on tadpole development (16). All these point to one possibility, *i. e.*, growth stimulated by antibiotics is not mediated through changes in the bacterial environment, but is accomplished instead *via* the endocrine glands. The results of the present experiments, although giving no direct evidence to the endocrine concept, definitely put antibacterial action of antibiotics in growth promotion out of place, for the growth of germ-free organisms was stimulated.

SUMMARY

1) Pregnant albino mice were injected intraperitoneally with aureomycin in the dosage of 0.2 mg per mouse per day during the whole gestation period. Four experiments were performed.

2) The repeated daily injection of aureomycin produced no deleterious effect on mice reproduction, for percentages of premature termination of pregnancy and still-birth were not influenced.

3) The litter size, length of gestation period, and increase of maternal body weight were not affected by aureomycin injection as compared with those of the controls.

4) Direct evidence of growth promoting action of aureomycin on mouse embryo was obtained in one out of four experiments, by the analysis of the birth weight. Indirect evidences were obtained in two additional experiments, by analyzing the correlation coefficient between birth weight and litter size, and the regression coefficient of birth weight on litter size.

5) It is concluded that the growth rate of germ-free intrauterine embryos was stimulated by injection of aureomycin to the mother, but the general condition of the mother was not affected.

6) This growth-stimulating effect is not a result of antibacterial action of the antibiotic, but is possibly due to the direct stimulation of aureomycin to endocrine glands.

REFERENCES

1. JUKES, T. H., and W. L. WILLIAMS. 1953. Nutritional effects of antibiotics. *Pharmacol. Rev.* **5:** 381-420.
2. STOKSTAD, E. J. R. 1945. Antibiotics in animal nutrition. *Physiol. Rev.* **34:** 25-51.
3. JUKES, T. H. 1955. *Antibiotics in nutrition.* Medical Encyclopedia, Inc., New York. p. 19-42.
4. CARPENTER, L. E. 1951. Effect of APF concentrate containing aureomycin on gestation, lactating and growing swine. *J. Animal Sci.* **10:** 657-664.
5. CARPENTER, L. E., and N. LARSON. 1953. Antibiotics and the reproduction of swine. *J. Animal Sci.* **12:** 812-818.
6. DAVEY, R. J., W. W. GREEN, and J. W. STEVENSON. 1955. The effect of aureomycin on growth and reproduction in swine. *J. Animal Sci.* **14:** 507-511.
7. SHUMWAY, W. 1942. *Introduction to vertebrate embryology.* Jonh Wiley & Sons, Inc., New York. 389 p.
8. WITSCHI, E. 1956. *Development of vertebrates.* W. B. Saunders Co., Philadelphia. 588 p.
9. ELLIOTT, R. W., and A. R. WHITEHILL. 1957. Placenta and mammary transfer of ingested chlortetracycline in the rat. *Proc. Soc. Exptl. Biol. Med.* **94:** 119-122.
10. MACDOWELL, E. C. E., E. ALLEN, and C. G. MACDOWELL. 1929. The relation of parity, age, and body weight to the number of corpora lutea in mice. *Anat. Record* **41:** 267-272.
11. HESTER, H. H. JR., F. R. LAUDAGORA, and L. L. RUSOFF. 1954. The distribution of aureomycin in the body of dairy calves showing a growth response when the antibiotic is administered orally or intramuscularly. *J. Animal Sci.* **13:** 988-989.
12. NICKELL, L. G. 1952. Stimulation of plant growth by antibiotics. *Proc. Soc. Exptl. Biol. Med.* **80:** 615-617.
13. BARBER, R. S., R. BRAUDE, and K. G. MITCHELL. 1953. Antibiotics and endocrine stimulants as promotors of growth in fattening pigs. *Chem. & Ind.* **17:** 410.
14. GRANT, W. C. 1954. Aureomycin and the thyroid gland. *Science* **120:** 724-725.
15. HSÜ, C. Y. 1960. The effect of aureomycin on development of the frog. *Embryologia* **5:** 321-334.
16. HSÜ, C. Y., C. M. PI, and H. M. LIANG. 1962. Effect of penicillin on frog development. *Embryologia* **7:** 179-183.